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(54) Title: RAPIDLY CLEARING TECHNETIUM-99m PHOSPHONATE SKELETAL IMAGING AGENTS

(57) Abstract

Preparations of radiolabelled bone scanning agents are improved by adding energy to the reconstituted kit in the form of heat. The preparations may be heated by autoclaving, boiling, microwaving, or sonicating. The improved agent exhibits more rapid soft tissue clearance which allows scanning of patients to be accomplished at shorter times after injection of the agent and reduces the radiation exposure to non-target tissues. These energy-modified formulations are also more stable than conventional formulations prepared at room temperature.

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RAPIDLY CLEARING TECHNETIUM-99m PHOSPHONATE SKELETAL IMAGING AGENTS

Background of the Invention

The present invention relates to technetium-99m, mono-, di-, and polyphosphonate complexes, to a method of preparation of the complexes, and to the radiopharmaceutical compositions of the complexes.

Recent work in nuclear medicine has been directed to technetium-99m (^{99m}Tc) which has a half-life of six hours. Interest in ^{99m}Tc also stems from the availability of convenient commercial means for supplying this radionuclide in the hospital as needed. A radionuclide solution in the oxidized pertechnetate ($^{99m}TcO_4^-$) form is obtained from commercial sources by elution with an isotonic saline solution from a commercially available ^{99m}Tc generator (for example, Mallinckrodt Medical, Inc. Ultra-Technekow UTK FM Tc-99m Generator). Technetium, as pertechnetate, from generators is in the +7 oxidation state, which does not combine with bone-seeking agents (mono-, di-, and polyphosphonates) to provide bone scans. This problem is easily overcome by reducing the pertechnetate to the +3, +4 and/or +5 oxidation state. Technetium-99m does not itself seek or react with the skeleton, but must first be complexed to an agent which does react with the skeleton.

In general, ^{99m}Tc bone scanning agents are prepared by mixing a pertechnetate-99m saline solution with a pertechnetate reducing agent in the presence of a bone-seeking agent to produce the radiolabelled complex. Kits containing the bone-seeking agent (generally, a diphosphonate) and a pertechnetate-99m reducing agent are readily available from commercial sources.

Current skeletal imaging procedures utilizing technetium-99m phosphonate formulations require that scintigraphic skeletal images be acquired after a minimum 2 to 4 hours to a maximum of 24 hours post-injection of the

agent. A major factor forcing the waiting period is allowing for sufficient clearance of the radiolabelled complex from the blood and soft tissue (muscle, liver, etc.) (Fogelman, I., ed., "Bone Scanning in Clinical Practice," Springer-Verlag, London, 1987). The extensive waiting period between injection of the agent and the scanning of the patient precludes rapid diagnosis of skeletal abnormalities by the attending physician and creates both patient, technician, and physician inconvenience.

The technetium-99m diphosphonate solutions consist of a complex mixture of components as identified by numerous peaks in the high performance liquid chromatogram (HPLC), indicating that the agent is not a single, pure species in solution (T.C. Pinkerton, W.R. Heineman, E. Deutsch, Anal. Chem., 52, 1106-1110, 1980). The various components of the technetium-99m diphosphonate formulations characterized and isolated by HPLC have dramatically different biodistribution patterns evidenced by different bone, blood, and soft tissue uptakes of the isolated species. The complex composition of the technetium-99m diphosphonate formulations can be altered by heating as determined by HPLC characterization of the formulation (T.C. Pinkerton, D.L. Ferguson, E. Deutsch, W.R. Heineman, K. Libson, Int. J. Appl. Radiat. Isot., 33, 907-915, 1982; S.C. Srivastava, G.E. Meinken, P. Richards, L.A. Ford, W.R. Benson, "Third World Congress of Nuclear Medicine and Biology", Paris, 1982, pp. 1631-1634). Therefore, the position in the HPLC chromatogram of a peak corresponding to a component in the formulation can be correlated with the biodistribution of that component.

Thus, there is a need in the art for technetium-99m mono-, di- or polyphosphonate compositions which clear more rapidly from the blood and soft tissue to allow scanning of the patient in less than 4 hours post-injection and to lower the radiation dose to non-target tissues.

Such phosphonate compositions for diagnosis and therapy are disclosed and claimed herein.

Summary of the Invention

Preparations of radiolabelled bone scanning agents can be improved by adding energy, in the form of heat, to a reconstituted kit containing a phosphonate ligand, ^{99m}Tc pertechnetate solution, a reducing agent, and optionally a stabilizer. The added energy improves the bone scanning agent by improving blood and soft tissue clearance. This allows patients to be scanned at shorter times after injection of the agent and lowers the radiation dose to non-target tissues. The bone scanning agent may be heated within the scope of the present invention using such methods as an autoclave, boiling water bath, microwave oven, or ultrasonic bath. Combinations of the foregoing methods may also be use.

BRIEF DESCRIPTION OF THE DRAWINGS

In order that the manner in which the above-recited and other advantages and features of the invention are obtained, a more particular description of the invention briefly described above will be rendered by reference to specific embodiments thereof which are illustrated in the appended drawings. Understanding that these drawings depict only typical embodiments of the invention and are not therefore to be considered limiting of its scope, the invention will be described and explained with additional specificity and detail through the use of the accompanying drawings in which:

Figure 1 shows the HPLC of ^{99m}Tc-HMDP kit at room temperature, pH 4.2.

Figure 2 shows the HPLC of ^{99m}Tc-HMDP kit autoclaved for 60 minutes, pH 4.2.

Figure 3 shows the HPLC of ^{99m}Tc-HMDP kit autoclaved for 60 minutes at pH 2.5-3.0.

Figure 4 shows the HPLC of ^{99m}Tc-MDP Kit prepared at room temperature.

Figure 5 shows the HPLC of ^{99m}Tc-MDP kit autoclaved for 60 minutes, pH 6.85.

Figure 6 shows the HPLC of ^{99m}Tc-MDP kit autoclaved for 60 minutes at pH 2.5.

Figure 7 shows the HPLC of ^{99m}Tc-HEDP Kit prepared at room temperature.

Figure 8 shows the HPLC of ^{99m}Tc-HEDP kit autoclaved for 60 minutes, pH 4.5.

Figure 9 shows the HPLC of ^{99m}Tc-HEDP kit autoclaved for 60 minutes at pH 2.5.

Detailed Description of the Preferred Embodiments

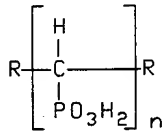
This invention can be fulfilled by a modification of any technetium-99m mono-, di-, or polyphosphonate formulation by (1) heating, (2) microwaving, (3) sonicating, (4) sonicating with heating, or (5) any other manipulation of the preparation conditions intended to effect a change in the composition of the components of the formulation of the radiolabelled phosphonate solutions to alter the composition of the mixture to produce species which will clear rapidly from the blood and soft tissue.

In its method aspects, the present invention includes a method of preparing bone scanning agents by heating the compositions and in some cases lowering the pH of the composition during the heating process.

The kit is comprised of pertechnetate ^{99m}Tc, a phosphonate, a reductant, and an optional stabilizer at about pH 1-10, preferably from pH 1-5, heated from 50°C-150°C for 5 minutes to 2 hours, preferably greater than 75°C for 5 to 60 minutes, or microwaved at 300-750 watts for 10 seconds to 5 minutes, preferably at 300-500 watts for 30 seconds to 2 minutes, or sonicated in an ultrasonic bath for 5 to 30 minutes, preferably with heating for 5 to 15 minutes. The foregoing time periods

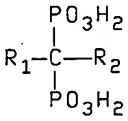
are based upon a typical imaging agent volume of about 10 mL. Those skilled in the art will appreciate that for smaller volumes, the time periods may be reduced. These temperature-modified formulations are also more stable than the formulations prepared at room temperature based upon changes in HPLC over time.

A broad range of mono-, di- and polyphosphonic acids and their pharmaceutically acceptable salts are now known to concentrate on the skeleton upon injection of solutions thereof into a patient. Operable species for this purpose include mono-, di- and polyphosphonates selected from the group consisting of:



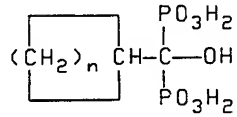
(I)

wherein each R is hydrogen or CH_2OH and n is an integer of from 3 to 10;



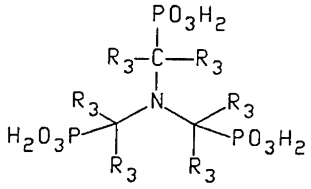
(II)

wherein R_1 is hydrogen, alkyl containing from 1 to about 20 carbon atoms, alkenyl containing from 2 to about 20 carbon atoms, aryl (e.g., phenyl, naphthyl), phenylethenyl, benzyl, halogen (e.g., chlorine, bromine and fluorine), hydroxyl, amino, substituted amino (e.g., dimethylamino, diethylamino, N-hydroxy-N-ethylamino, acetylamino), $-CH_2COOH$, $-CH-_2PO_3H_2$, $CH(PO_3H_2)(OH)$, or $[CH_2C(PO_3H_2)_2]_n-H$ where n=1 to 15, R_2 is hydrogen, lower alkyl (e.g., methyl, ethyl, propyl and butyl), amino, benzyl, halogen (e.g., chlorine, bromine, and fluorine), hydroxyl, $-CH_2COOH$, $-CH_2PO_3H_2$, or $CH_2CH_2PO_3H_2$;



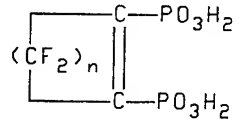
(III)

wherein n is an integer of from 3 to 9;



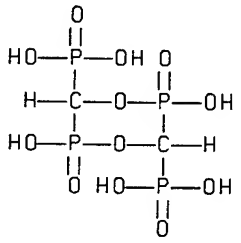
(IV)

wherein each R_3 is hydrogen or lower alkyl (e.g., methyl, ethyl, propyl and butyl);

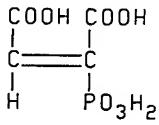


(V)

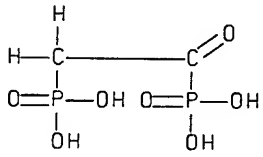
wherein n is an integer of from 2 to 4;



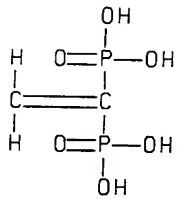
(VI)



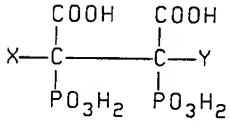
(VII)



(VIII)



(IX)



(X)

wherein X and Y are each hydrogen or hydroxy; and the non-toxic salts of each of the foregoing phosphonates which in an essentially neutral aqueous solution will react with hereinafter enumerated reducing/complexing materials; i.e., stannous, ferrous, or chromous salts to form the corresponding stannous, ferrous or chromous phosphonate salt. Suitable reactive phosphonate salts (hereinafter referred to as pharmaceutically acceptable salts) for use with the present invention include sodium, potassium, ammonium and low molecular weight substituted ammonium (e.g., mono-, di and tri-ethanolamine and quaternary ammonium) salts of the above phosphonates and mixtures thereof.

Operable polyphosphonates of the above formula (I) include propane-1,2,3-triphosphonic acid; butane-1,2,3,4-tetraphosphonic acid; hexane-1,2,3,4,5,6-hexaphosphonic acid; hexane-1-hydroxy-2,3,4,5,6-pentaphosphonic acid; hexane-1,6-dihydroxy-2,3,4,5-tetraphosphonic acid; pentane-1,2,3,4,5-pentaphosphonic acid; heptane-1,2,3,4,5,6,7-heptaphosphonic acid; octane-1,2,3,4,5,6,7,8-octaphosphonic acid, nonane-1,2,3,4,5,6,7,8,9-nonaphosphonic acid; decane-1,2,3,4,5,6,7,8,9,10-decaphosphonic acid; and the pharmaceutically acceptable salts of these acids e.g., sodium, potassium, ammonium, triethanolammonium, diethanolammonium, and monoethanolammonium salts.

Propane-1,2,3-triphosphonic acid and salts thereof can be prepared by a process disclosed in U.S. Pat. No. 3,743,688 to D. Allan Nicholson and Darrel Campbell.

Butane-1,2,3,4-tetraphosphonic acid and salts thereof can be prepared by a process disclosed in U.S. Pat. No. 3,755,504 to D. Allan Nicholson and Darrel Campbell.

The higher aliphatic vicinal polyphosphonates and salts thereof can be prepared by the process disclosed in U.S. Pat. No. 3,584,035 granted June 8, 1971.

Among the operable polyphosphonates encompassed by the above formula (II) are ethane-1-hydroxy-1,1-diphosphonic acid; methanediphosphonic acid; methanehydroxydiphosphonic acid; ethane-1,1,2-triphosphonic acid; ethane-2-phenyl-1,1-diphosphonic acid; ethane-2-naphthyl-1,1-diphosphonic acid; methanephenyldiphosphonic acid; ethane-1-amino-1,1-diphosphonic acid; methanedichlorodiphosphonic acid; nonane-5,5-diphosphonic acid; n-pentane-1,1-diphosphonic acid; methanedifluorodiphosphonic acid; methanedibromodiphosphonic acid; propane-2,2-diphosphonic acid; ethane-2-carboxy-1,1-diphosphonic acid; propane-1-hydroxy-1,1,3-triphosphonic acid; ethane-2-hydroxy-1,1,2-triphosphonic acid; ethane-1-hydroxy-1,1,2-triphosphonic acid; propane-1,3-diphenyl-2,2-diphosphonic acid; nonane-1,1-diphosphonic acid; hexadecane-1,1-diphosphonic acid; pent-4-ene-1-hydroxy-1,1-diphosphonic acid; octadec-9-ene-1-hydroxy-1,1-diphosphonic acid; 3-phenyl-1,1-diphosphono-prop-2-ene; octane-1,1-diphosphonic acid; dodecane-1,1-diphosphonic acid; phenylaminomethanediphosphonic acid; naphthylaminomethanediphosphonic acid; N,N-dimethylaminomethanediphosphonic acid; N-(2-dihydroxyethyl)-aminomethanediphosphonic acid; N-acetylaminomethanediphosphonic acid; aminomethanediphosphonic acid; dihydroxymethanediphosphonic acid; and the pharmaceutically acceptable salts of these acids, e.g., sodium, potassium, ammonium, triethanolammonium, diethanolammonium, and monoethanolammonium salts.

Ethane-1-hydroxy-1,1-diphosphonic acid, an especially preferred polyphosphonate, has the molecular formula $CH_3C(OH)PO_3H_2)_2$. (According to nomenclature by radicals, the acid might also be named 1-hydroxyethylidene diphosphonic acid).

While any pharmaceutically acceptable salt of ethane-1-hydroxy-1, 1-diphosphonic acid can be used in the practice of this invention, mixtures of the disodium and trisodium salts are most preferred. To other sodium, potassium, ammonium, and mono-, di-, and triethanolammonium salts and mixtures thereof are also suitable, provided caution is observed in regulating the total intake of cation species in the salt composition. These compounds can be prepared by any suitable method, however, an especially preferred method is disclosed in U.S. Patent No. 3,400,149 granted Sept. 3, 1968.

Methanehydroxydiphosphonic acid and related compounds operable herein can be prepared, for example, by reaction of phosgene with an alkali metal dialkylphosphite. A complete description of these compounds and a method for preparing same is found in U.S. Pat. No. 3,422,137 granted Jan. 14, 1969.

Methanedihydroxydiphosphonic acid and salts useful herein and a method for preparing same are disclosed in U.S. Pat. No. 3,497,313 granted Feb. 24, 1970.

Methanediphosphonic acid and related compounds useful herein are described in detail in U.S. Pat. No. 3,213,030, granted Oct. 19, 1965. A preferred method of preparing such compounds is disclosed in U.S. Pat. No. 3,251,907 granted May 17, 1966.

Ethane-1,1,2-triphosphonic acid and related compounds which can be used in the compositions of this invention, as well as a method for their preparation, are fully described in U.S. Pat. No. 3,551,339 granted Dec. 29, 1970.

Propane-1,1,3,3-tetraphosphonic acid and related compounds useful herein, and a method for preparing same

are fully disclosed in U.S. Pat. No. 3,400,176 granted Sept. 3, 1968. The higher methylene interrupted methylene diphosphonate polymers can be prepared by the polymerization of ethylene-1,1-diphosphonate.

Pentane-2,2-diphosphonic acid and related compounds can be prepared in accordance with the method described by G.M. Kosolopoff in J. Amer. Chem. Soc., 75, 1500 (1953).

Operable phosphonates of formula (III) above include the following:

Methanecyclobutylhydroxydiphosphonic acid
Methanecyclopentylhydroxydiphosphonic acid
Methanecyclohexylhydroxydiphosphonic acid
Methanecycloheptylhydroxydiphosphonic acid
Methanecyclooctylhydroxydiphosphonic acid
Methanecyclononylhydroxydiphosphonic acid
Methanecyclodecylhydroxydiphosphonic acid

Each of the sodium, potassium, ammonium, monoethanol-ammonium, diethanolammonium, and triethanolammonium salts of the above recited methanecycloalkylhydroxydiphosphonic acids as well as any other pharmaceutically acceptable salt of these acids, all selectively seek the skeleton.

The phosphonates of formula (III) can be prepared by methods fully described in U.S. Pat. No. 3,584,125, granted June 8, 1971.

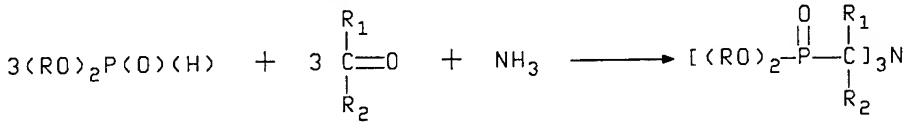
The preferred phosphonates of formula (IV) for the purpose of this invention are tris(phosphonomethyl)mine; tris(1-phosphonoethyl)amine; tris(2-phosphonopropyl)amine; and their pharmaceutically acceptable salts. Tris(phosphonomethyl)amine is especially preferred. The following are exemplary of compounds which can also be used:

(a) bis(phosphonomethyl)-1-phosphonoethyl amine;
(b) bis(phosphonomethyl)-2-phosphono-2-propamine;
(c) bis(1-phosphonomethyl)phosphonomethyl amine;
(d) bis(2-phosphono-2-propyl)phosphonomethyl amine
(e) tris(1-phosphono-1-pentyl)amine;
(f) bis(phosphonomethyl)2-phosphono-2-hexyl amine;

and

(g) the pharmaceutically acceptable salts of acids (a) through (f), e.g., sodium, potassium, ammonium triethanolammonium, diethanolammonium, and monoethanolammonium salts.

The tris(phosphonoalkyl)amines can be prepared, for example, by first preparing the corresponding ester accordance with the general reaction:



wherein R is alkyl and R_1 and R_2 are hydrogen or low alkyl.

The free acids can be prepared by hydrolysis of ester using strong mineral acids such as hydrochloric acid. The salts, are, of course prepared by neutralizing the acid with the base of the desired cation. The preparation of tris(phosphonoalkyl)amines is fully disclosed by Irani, et al., in Canadian Pat. No. 753,207, issued Feb 21, 1967.

The phosphonates of formula (V) include the following;

(1) 3,3,4,4,5,5-hexafluoro-1,2-diphosphonocyclopent-1-ene;

(2) 3,3,4,4,-tetrafluoro-1,2-diphosphonocyclobut-1-ene; and

(3) 3,3,4,4,5,5,6,6-octafluoro-1,2-diphosphonocyclohex-1-ene.

The perfluorodiphosphonocycloalkenes can be prepared, for example, by reacting trialkyl phosphites with 1,2-dichloroperfluorocycloalk-1-enes in accordance with the procedures fully described by Frank in J. O. Chem., 31, #5, p. 1521.

The phosphonates of formula (VI) is referred to herein as cyclic tetraphosphonic acid. This compound and its pharmaceutically acceptable salts can be prepared by any suitable method, however, an especially preferred method is disclosed by Oscar T. Quimby, U.S. Pat. No. 3,387,024 granted June 4, 1968.

Operable phosphonates encompassed by the above formula (VII) are ethane-1,2-dicarboxy-1-phosphonic acid; and the

pharmaceutically acceptable salts of these acids, e.g., sodium potassium, ammonium, triethanolammonium, diethanolammonium, and monoethanolammonium salts. While the above formula (VII) is representative of cis-isomers, the corresponding trans-isomers are also useful herein. Reference hereinafter to ethane-1,2-dicarboxy-1-phosphonic acid or salts thereof, unless otherwise specified, is intended as contemplating the cis- and trans-isomers and mixtures thereof.

Ethane-1,2-dicarboxy-1-phosphonic acid and related compounds useful herein can be prepared by reaction of an ester of acetylenedicarboxylic acid and a dialkyl phosphite followed by hydrolysis and saponification. This method is more fully described in U.S. Patent No. 3,584,124, granted June 8, 1971.

The sodium salt of formula (VIII) can be made by the rearrangement reaction of a 2-haloethane-1-hydroxy-1,1-diphosphonic acid with about 3 equivalents of sodium hydroxide as disclosed in U.S. Patent No. 3,641,126.

The phosphonate of formula (IX) can be made by the method of German Offenlegunsschrift No. 2,076,078.

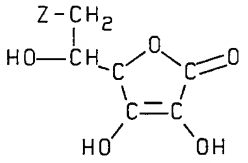
Operable carboxyphosphonates of the above formula (X) include ethane-1,2-dicarboxy-1,2-diphosphonic acid; ethane-1,2-dicarboxy-1,2-dihydroxy-1,2-diphosphonic acid; ethane-1,2-dicarboxy-1-hydroxy-1,2-diphosphonic acid; and the pharmaceutically acceptable salts of these acids, e.g., sodium, potassium, ammonium, triethanolammonium, diethanolammonium and monoethanolammonium salts.

Ethane-1,2-dicarboxy-1,2-diphosphonic acid, a preferred carboxyphosphonate herein, has the molecular formula $CH(COOH)(PO_3H_2)CH(COOH)(PO_3H_2)$. The most convenient crystallizable salts of this acid are obtained when three, four or five of the acid hydrogens are replaced by sodium.

While any pharmaceutically acceptable salt of ethane-1,2-dicarboxy-1,2-diphosphonic acid can be used in the practice of this invention, the tetrasodium dihydrogen

atom; lower alkenyl (containing from 2 to about 8 carbon atoms); nicotinic acid and nicotinamide complexes thereof and pharmaceutically-acceptable salts, esters, and amides thereof. Syntheses of these compounds are described in the following literature, incorporated by reference herein; Bock, et al., Carbohydrate Research, 68, 313-319 (1979); Cousins, et al., Journal of the American Oil Chemists Society, 54, 308-312 (1977); Feather, et al., Journal of the Organic Chemistry, 31, 4018-4021 (1966); and Wenner, Journal of Organic Chemistry, 14, 22-26 (1949).

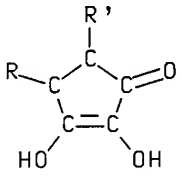
Preferred compounds of formula (XI) include halogen-substituted ascorbic acids of the formula:



(XII)

wherein Z is halogen. Compounds of this formula (XII) include 6-bromo-6-deoxyascorbic acid, 6-chloro-6-deoxyascorbic acid, and 6-iodo-6-deoxyascorbic acid.

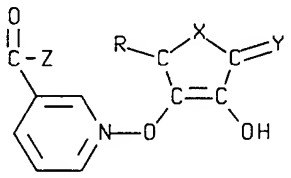
Another class of preferred compounds of formula XI include compounds of the formula:



(XIII)

wherein R and R' are as defined above. Compounds of this formula (XIII) include reductic acid, 4-methyl reductic acid, 5-ethyl reductic acid, 5-methyl reductic acid, and 5-ethyl reductic acid.

A third group of preferred compounds include the nicotinamide complexes of compounds of formula (XI); i.e.:



(XIV)

wherein X, Y, and R are as defined above, and Z is OH or NH_2. Compounds of this formula XIV include nicotinic acid and nicotinamide complexes of 6-bromo-6-deoxyascorbic acid, 6-chloro-6-deoxyascorbic acid, reductic acid, and 5-methylreductic acid.

Preferred compounds of the formulae above have particular advantages over stabilizers currently known and used in the art. For example, 6-bromo-6-deoxyascorbic acid is a more effective stabilizer than ascorbic acid or erythorbic acid. (Ascorbic acid and erythorbic acid are stabilizers analogous to formula (XII), above, wherein "Z" is hydroxyl; as described in German Offenlegungsschrift No. 2,618,337, Tofe, published Nov. 11, 1976.)

In practice, the salt and ester forms of reductate stabilizers suitable for use in the present invention can be selected for use according to their solubility in a pertechnetate solution. It is, of course, preferable that the salts and esters be readily soluble in a pertechnetate solution. Accordingly, suitable salts include the alkali metal, alkaline earth metal, heavy metal and ammonium salts. The alkali metal salts such as sodium, potassium and lithium salts are readily soluble and accordingly preferred for use herein. Various ammonium salts, wherein the cation is $N(R')_4$ are also suitable for use herein. These include, for example, alkylammonium, alkanolammonium and arylammonium salts. It is of course, understood that the solubility of ammonium salts is largely dependent upon the number and nature of the substituent groups on the nitrogen atom. In general, and as used herein, preferred readily soluble ammonium salts include those wherein each R' is either hydrogen or C_1 to about C_5 hydrocarbyl. Nonlimiting examples of pharmaceutically-acceptable ammonium salts useful herein include the ammonium, methyl-ammonium, dimethylammonium, tetramethylammonium, bis-(tetramethylammonium). 2-hydroxypropylammonium, bis-(2-hydroxypropylammonium), ethanolammonium, diethanolammonium,

salt, the trisodium trihydrogen salt, the disodium tetrahydrogen salt, the monosodium pentahydrogen salt, and the mixtures thereof are useful. The other potassium, ammonium, and mono-, di-, and triethanolammonium, etc., salts and mixtures thereof are also suitable provided caution is observed in regulating the total intake of cation species in the salt composition.

Ethane-1,2-dicarboxy-1,2-diphosphonic acid and suitable salts thereof can be prepared in any convenient manner. For example, the reaction described by Pudovik in "Soviet Research on Organo-Phosphorus Compounds", 1949-1956, Part III, 547-85c. can be used to prepare the ester of ethane-1,2-dicarboxy-1,2-diphosphonic acid which in turn can, by ordinary hydrolysis reactions, be converted to the free acid form. Neutralization by alkali compounds such as sodium hydroxide, potassium hydroxide, carbonates and the like can be used to prepare a desired salt of the acid. A more detailed description of the preparation of these compounds is described in U.S. Pat. No. 3,562,166 granted Feb. 9, 1971.

Ethane-1,2-dicarboxy-1,2-dihydroxy-1,2-diphosphonic acid and related compounds useful herein can be prepared by reaction of an ester of ethane-1,2-dicarboxy-1,2-diphosphonic acid and an alkali metal hypohalite followed by hydrolysis and saponification. This method is more fully described in U.S. Pat. No. 3,579,570 granted May 18, 1971.

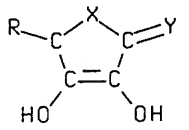
Mixtures of any of the foregoing phosphonic acids and/or salts can be used in the practice of this invention.

Currently preferred phosphonic acids and/or salts for use within the scope of the present invention include methanediphosphonic acid (MDP), Methanehydroxydiphosphonic acid (HMDP), ethane-1-hydroxy-1,1-diphosphonic acid (HEDP), N,N-dimethylaminomethanediphosphonic acid (DMAD), propane-2,3-dicarboxy-1,1-diphosphonic acid (DAD), and pharmaceutically acceptable salts thereof.

Suitable pertechnetate reducing agents include metal salts of sulfuric acid and hydrochloric acid, such as stannous chloride, chromous chloride, cuprous chloride, and ferrous sulfate. Other agents capable of reducing pertechnetate include, for example, cuprous and ferrous salts with ascorbic acid or salts of ascorbic acid, titanous halides, acid-thiosulfates, acid-hydrogen-sulfates, salts of sulfites, salts of bisulfites, acid-bisulfites, salts of dithionites, acid dithionites, acid-sulfites, iron colloids, acid borohydrides, salts of phosphites, acid-phosphites, salts of hypophosphites, acid-hypophosphites, salts of molybdenum(III), salts of nitrite, hydrazines, dithiothreitol, hydroxylamines, dihydroxy-benzene derivatives, dinitrobenzene derivatives, salts of sulfinic acids, sulfinic acids, electrodes, acid-nitrites. Water soluble stannous (Sn^{2+}) compounds, especially stannous chloride, are preferred for use as the pertechnetate reducing agent.

Suitable stabilizing agents include ascorbic acid and water soluble salts and esters of ascorbic acid, gentisic acid and water soluble salts and esters of gentisic acid, hydroquinone, erythorbic acid and water soluble salts and esters of erythorbic acid, and reductate stabilizers.

Reductate stabilizers are compounds and mixtures of compounds of the formula:



(XI)

wherein X is CRR', O, or NR' is hydrogen, or lower alkyl (containing 1 to 8 carbon atoms), Y is oxygen, sulfur, nitrogen or CH_2; R is hydrogen, lower alkyl containing from 1 to 8 carbon atoms, alkyl containing from 3 to 8 carbon atoms substituted with one or more hydroxy, halogen, amino or thiol groups, lower alkyl containing from 1 to 8 carbon atoms halogen-substituted on the first and/or second carbon

triethanolammonium, bis-(triethanolammonium), phenylammonium, naphthylammonium and quinolylammonium salts.

The alkaline earth metal salts, for example the calcium and magnesium salts, although less soluble, are also suitable for use herein. The heavy metal salts, for example the iron and tin salts, are also suitable for use herein.

The pharmaceutically-acceptable esters of the reductate stabilizers, readily soluble in pertechnetate solutions, include, for example, the C_1 to C_{20} alkyl esters such as the methyl, ethyl, propyl, isopropyl, butyl, isobutyl, pentyl, hexyl, octyl, and palmityl esters.

In embodiments of this invention in which the reductate stabilizers is combined with the pertechnetate reductant, the choice of reductant is not critical. As used herein the term "pertechnetate reductant" is intended to include compounds, complexes, or the like, comprising a reducing ion capable of reducing heptavalent technetium (TcO_4^-) to trivalent, tetravalent and/or pentavalent technetium. Free metals such as tin are also known for use as pertechnetate reductants, although undissolved metal must be removed from the imaging solution prior to infection into the patient. Thus, it is more convenient to use metal compounds which provide the reducing-metal cation in soluble form.

The following examples are offered to further illustrate the present invention. These examples are intended to be purely exemplary and should not be viewed as a limitation on any claimed embodiment.

EXAMPLE 1

Preparation of a ^{99m}Tc-HMDP Kit

An Osteoscan® HDP kit (Mallinckrodt Medical, Inc.) was reconstituted to 3 mL with a combination of saline and pertechnetate solution eluted from a ^{99m}Tc generator (MMI) in accordance with the package insert. The solution was

allowed to stand for 5 minutes at room temperature. Analysis of the solution by high performance liquid chromatography (HPLC) (μ-Bondapak C-18 column, Waters Associates, 300 x 4 mm) showed the presence of several species upon elution with mobile phase consisting of 10% dioxane, 0.75 mM tetrabutyl ammonium hydroxide, 1 mM sodium acetate, 2.5 mM disodium oxidronate (HMDP), pH 5.2 at a flow rate of 2.0 mL/min. The resulting chromatogram indicates the complex nature of the ^{99m}Tc-HMDP solution. Figure 1 shows the HPLC of ^{99m}Tc-HMDP kit at room temperature, pH 4.2.

A second HPLC was performed after allowing the solution to sit at room temperature for 24 hours. The resulting HPLC chromatogram was different than the earlier chromatogram indicating a change in the composition and distribution of ^{99m}Tc-HMDP oligomers/polymers in the reaction mixture.

EXAMPLE 2

Preparation of an Autoclaved ^{99m}Tc-HMDP Kit

A second Osteoscan® HDP kit was prepared as in Example 1. However, the reconstituted vial was autoclaved (121°C, 15 psi) for 60 minutes. The solution was allowed to cool to room temperature and then analyzed by HPLC chromatography as in Example 1. The resulting chromatogram shows a single peak which elutes later than the collection of peaks shown in Example 1, indicating a change in the composition and distribution of ^{99m}Tc-HMDP oligomers/polymers in the reaction mixture as evidenced by HPLC analysis. Figure 2 shows the HPLC of ^{99m}Tc-HMDP kit autoclaved for 60 minutes, pH 4.2.

A second HPLC was performed after allowing the solution to sit at room temperature for 24 hours. The resulting HPLC chromatogram was essentially identical to the earlier chromatogram indicating that the ^{99m}Tc-HMDP oligomers/polymers in the reaction mixture were stable.

EXAMPLE 3

Preparation of an Autoclaved ^{99m}Tc-HMDP Kit at pH 2

A third Osteoscan® HDP kit was prepared as in Example 1 except that 40 μL of 1.0 N hydrochloric acid (HCl) was added to the reconstituted vial to decrease the pH to about pH 2. The pH 2 solution was autoclaved for 60 minutes as in Example 2. The solution was allowed to cool to room temperature and analyzed by HPLC chromatography as in Example 1. The resulting chromatogram shows primarily a single peak which elutes early compared to either Example 1 or Example 2, indicating a change in the composition and distribution of ^{99m}Tc-HMDP oligomers/polymers in the reaction mixture as evidenced by HPLC analysis. Figure 3 shows the HPLC of ^{99m}Tc-HMDP kit autoclaved for 60 minutes at pH 2.5-3.0.

A second HPLC was performed after allowing the solution to sit at room temperature for 24 hours. The resulting HPLC chromatogram was essentially identical to the earlier chromatogram indicating that the ^{99m}Tc-HMDP oligomers/polymers in the reaction mixture were stable.

From Examples 1-3, it will be appreciated that the pH and temperature conditions can be altered to produce a bone scanning agent which clears more rapidly from the soft tissue than the currently available agents.

EXAMPLE 4

Preparation of a ^{99m}Tc-MDP Kit

A Technescan® MDP kit (Merck-Frosst, sold by Mallinckrodt Medical, Inc.) was reconstituted to 5 mL with a combination of isotonic saline and pertechnetate solution eluted from a Tc-99m generator (MMI) in accordance with the package insert. The pH 6.85 solution was allowed to stand for 5 minutes at room temperature. Analysis of the solution by high performance liquid chromatography (HPLC) (μ-Bondapak C-18 column, Waters Associates, 300 x 4 mm) showed the presence of several species upon elution with

mobile phase consisting of 10% dioxane, 1 mM tetrabutyl ammonium hydroxide, 1 mM sodium acetate, 2.5 mM medronic acid (MDP), pH 5.2 at a flow rate of 1.5 mL/min. The resulting chromatogram indicates the complex nature of the ^{99m}Tc-MDP solution. Figure 4 shows the HPLC of ^{99m}Tc-MDP Kit prepared at room temperature.

EXAMPLE 5

Preparation of an Autoclaved ^{99m}Tc-MDP Kit

A second Technescan® MDP kit was prepared as in Example 4. However, the reconstituted vial was autoclaved (121°C, 15 psi) for 60 minutes. The solution was allowed to cool to room temperature and then analyzed by HPLC chromatography as in Example 4. The resulting chromatogram shows a broad late eluting peak as well as a substantial amount of peaks corresponding to the peaks in the room temperature preparation shown in Example 4. Figure 5 shows the HPLC of ^{99m}Tc-MDP kit autoclaved for 60 minutes, pH 6.85.

EXAMPLE 6

Preparation of an Autoclaved ^{99m}Tc-MDP Kit at pH 2.5

A third Technescan® MDP kit was prepared as in Example 4 except that 85 μL of 1.0 N hydrochloric acid (HCl) were added to the reconstituted vial to decrease the pH to about pH 2.5. The pH 2.5 solution was autoclaved for 60 minutes as in Example 2. The solution was allowed to cool to room temperature and analyzed by HPLC chromatography as in Example 4. The resulting chromatogram shows primarily a single peak which elutes early compared to either Example 4 or Example 5, indicating a change in the composition and distribution of ^{99m}Tc-MDP oligomers/polymers in the reaction mixture as evidenced by HPLC analysis. Figure 6 shows the HPLC of ^{99m}Tc-MDP kit autoclaved for 60 minutes at pH 2.5.

EXAMPLE 7

Preparation of Lyophilized Kits containing HEDP

Lyophilized (freeze-dried) kits were prepared, each containing 5.9 mg of disodium etidronate (HEDP), 0.19 mg of stannous chloride dihydrate (pertechnetate reductant), and 0.56 mg of gentisic acid (stabilizer). The pH of this formulation is about pH 4.5 upon reconstitution with 5 mL of isotonic saline.

EXAMPLE 8

Preparation of a ^{99m}Tc-HEDP Kit

An HEDP kit prepared in Example 7 was reconstituted to 5 mL with a combination of isotonic saline and pertechnetate solution eluted from a ^{99m}Tc generator (MMI). The pH 4.5 solution was allowed to stand for 5 minutes at room temperature. Analysis of the solution by HPLC (μ-Bondapak C-18 column, Waters Associates, 300 x 4 mm) showed the presence of several species upon elution with mobile phase consisting of 10% dioxane, 0.5 mM tetrabutyl ammonium hydroxide, 1 mM sodium acetate, 2.5 mM disodium etidronate (HEDP), pH 5.2 at a flow rate of 2.0 mL/min. The resulting chromatogram indicates the complex nature of the ^{99m}Tc-HEDP solution. Figure 7 shows the HPLC of ^{99m}Tc-HEDP kit prepared at room temperature.

EXAMPLE 9

Preparation of an Autoclaved ^{99m}Tc-HEDP Kit

An HEDP kit was prepared as in Example 8. However, the reconstituted vial was autoclaved (121°C, 15 psi) for 60 minutes. The solution was allowed to cool to room temperature and then analyzed by HPLC chromatography as in Example 8. The resulting chromatogram shows a single late eluting peak compared to the chromatogram in Example 8, indicating a change in the composition and distribution of ^{99m}Tc-HDP oligomers/polymers in the reaction mixture as

evidenced by HPLC analysis. Figure 8 shows the HPLC of ^{99m}Tc-HEDP kit autoclaved for 60 minutes, pH 4.5.

EXAMPLE 10

Preparation of Lyophilized Kits containing HEDP (pH 2.5)

Lyophilized (freeze-dried) kits were prepared, each containing 5.9 mg of disodium etidronate (HEDP), 0.19 mg of stannous chloride dihydrate (pertechnetate reductant), and 0.56 mg of gentisic acid (stabilizer). The pH of the solution was adjusted to pH 2.0 prior to lyophilization with 1.0 N HCl. The pH of formulation is about pH 2.5 upon reconstitution with 5 mL of isotonic saline.

EXAMPLE 11

Preparation of an Autoclaved ^{99m}Tc-HEDP Kit at pH 2.5

An HEDP kit was prepared as in Example 10. The pH 2.5 reconstituted vial was autoclaved for 60 minutes as in Example 2. The solution was allowed to cool to room temperature and analyzed by HPLC chromatography as in Example 8. The resulting chromatogram shows several peaks which elute differently compared to either Example 8 or Example 9, indicating a change in the composition and distribution of ^{99m}Tc-HEDP oligomers/polymers in the reaction mixture as evidenced by HPLC analysis. Figure 9 shows the HPLC of ^{99m}Tc-HEDP kit autoclaved for 60 minutes at pH 2.5.

Example 12

Preparation of a Sonicated ^{99m}Tc-HMDP Kit at pH 1

An Osteoscan® HDP kit is prepared as in Example 1. The pH is adjusted to 1 with hydrochloric acid. The reconstituted vial is placed in a Sonicor Model SC-150TM ultrasonic bath with heat control for 5 minutes. The solution is allowed to cool to room temperature and is analyzed by HPLC chromatography as in Example 1. The resulting chromatogram shows primarily a single peak which elutes early compared to either Example 1 or Example 2

which suggests the composition and distribution of ^{99m}Tc-HMDP oligomers/polymers is similar to Example 3.

A second HPLC is performed after allowing the solution to sit at room temperature for 24 hours. The resulting HPLC chromatogram is essentially identical to the earlier chromatogram indicating that the ^{99m}Tc-HMDP oligomers/polymers in the reaction mixture are stable over time.

Example 13

Preparation of a Sonicated ^{99m}Tc-MDP Kit at pH 8

A Technescan® MDP kit is prepared as in Example 4. The pH is adjusted to 8 with sodium hydroxide. The reconstituted vial is placed in a Sonicor Model SC-150TM ultrasonic bath with heat control for 30 minutes. The solution is allowed to cool to room temperature and is analyzed by HPLC chromatography as in Example 4. The resulting chromatogram shows a broad late eluting peak as well as several peaks which elute similar to Example 4 which suggests the composition and distribution of ^{99m}Tc-MDP oligomers/polymers is similar to Example 5.

Example 14

Preparation of a Sonicated ^{99m}Tc-HEDP Kit at pH 4.5

An HEDP kit is prepared as in Example 8. The reconstituted vial is placed in a Sonicor Model SC-150TM ultrasonic bath with heat control for 15 minutes. The solution is allowed to cool to room temperature and is analyzed by HPLC chromatography as in Example 8. The resulting chromatogram shows primarily a single peak which elutes later than Example 8 which suggests the composition and distribution of ^{99m}Tc-HEDP oligomers/polymers is similar to Example 9.

Example 15

Preparation of a Microwaved ^{99m}Tc-HMDP Kit at pH 4.2

An Osteoscan® HDP kit is prepared as in Example 1. The reconstituted vial is placed in a microwave oven having a power rating of 750 watts for 30 seconds. The solution is allowed to cool to room temperature and is analyzed by HPLC chromatography as in Example 1. The resulting chromatogram shows primarily a single peak which elutes later than Example 1 which suggests the composition and distribution of ^{99m}Tc-HMDP oligomers/polymers is similar to Example 2.

Example 16

Preparation of a Microwaved ^{99m}Tc-MDP Kit at pH 2

A Technescan® MDP kit is prepared as in Example 4. The pH is adjusted to 2 with hydrochloric acid. The reconstituted vial is placed in a microwave oven having a power rating of 500 watts for 2 minutes. The solution is allowed to cool to room temperature and is analyzed by HPLC chromatography as in Example 4. The resulting chromatogram shows primarily a single peak which elutes early compared to Example 4 which suggests the composition and distribution of ^{99m}Tc-MDP oligomers/polymers is similar to Example 6.

A second HPLC is performed after allowing the solution to sit at room temperature for 24 hours. The resulting HPLC chromatogram is essentially identical to the earlier chromatogram indicating that the ^{99m}Tc-MDP oligomers/polymers in the reaction mixture are stable over time.

Example 17

Preparation of a Microwaved ^{99m}Tc-HEDP Kit at pH 1

An HEDP kit is prepared as in Example 8. The pH is adjusted to 1 with hydrochloric acid. The reconstituted vial is placed in a microwave oven having a power rating of 300 watts for 5 minutes. The solution is allowed to cool

to room temperature and is analyzed by HPLC chromatography as in Example 8. The resulting chromatogram shows several peaks which elute early compared to Example 8 which suggests the composition and distribution of ^{99m}Tc-HEDP oligomers/polymers is similar to Example 11.

Example 18

Preparation of a Boiled ^{99m}Tc-HMDP Kit at pH 7

An Osteoscan® HDP kit is prepared as in Example 1. The pH is adjusted to 7 with sodium hydroxide. The reconstituted vial is placed in a boiling water bath for 60 minutes. The solution is allowed to cool to room temperature and is analyzed by HPLC chromatography as in Example 1. The resulting chromatogram shows primarily a single peak which elutes late compared to Example 1 which suggests a change in the composition and distribution of ^{99m}Tc-HMDP oligomers/polymers in the reaction mixture as evidenced by HPLC analysis.

Example 19

Preparation of a Boiled ^{99m}Tc-MDP Kit at pH 5

A Technescan® MDP kit is prepared as in Example 4. The pH is adjusted to 5 with hydrochloric acid. The reconstituted vial is placed in a boiling water bath for 10 minutes. The solution is allowed to cool to room temperature and is analyzed by HPLC chromatography as in Example 4. The resulting chromatogram shows a collection of peaks which elute early compared to Example 4 which suggests a change in the composition and distribution of ^{99m}Tc-MDP oligomers/polymers in the reaction mixture as evidenced by HPLC analysis.

Example 20

Preparation of a Boiled ^{99m}Tc-HEDP Kit at pH 9

An HEDP kit is prepared as in Example 8. The pH is adjusted to 9 with sodium hydroxide. The reconstituted

vial is placed in a boiling water bath for 120 minutes. The solution is allowed to cool to room temperature and is analyzed by HPLC chromatography as in Example 8. The resulting chromatogram shows a single late eluting peak compared to Example 8 which suggests a change in the composition and distribution of ^{99m}Tc-HEDP oligomers/polymers in the reaction mixture as evidenced by HPLC analysis.

A second HPLC is performed after allowing the solution to sit at room temperature for 24 hours. The resulting HPLC chromatogram is essentially identical to the earlier chromatogram indicating that the ^{99m}Tc-HEDP oligomers/polymers in the reaction mixture are stable over time.

Example 21

Administration and Imaging of Autoclaved ^{99m}Tc-HMDP

An Osteoscan® HDP kit is prepared as in Example 3. The ^{99m}Tc-HMDP is administered to a patient at a dosage of 10 mCi. Scintigraphic skeletal images of the patient are obtained approximately one hour post injection. The skeletal images are of good quality and suggest that the ^{99m}Tc-HMDP cleared rapidly from blood and soft tissue.

Example 22

Administration and Imaging of Sonicated ^{99m}Tc-MDP

A Technescan® MDP kit is prepared as in Example 13. The ^{99m}Tc-MDP is administered to a patient at a dosage of 15 mCi. Scintigraphic skeletal images of the patient are obtained approximately one hour post injection. The skeletal images are of good quality and suggest that the ^{99m}Tc-MDP cleared rapidly from blood and soft tissue.

Example 23

Administration and Imaging of Microwaved ^{99m}Tc-HEDP

An HEDP kit is prepared as in Example 17. The ^{99m}Tc-HEDP is administered to a patient at a dosage of 20 mCi. Scintigraphic skeletal images of the patient are

obtained approximately two hours post injection. The skeletal images are of good quality and suggest that the ^{99m}Tc-HEDP cleared rapidly from blood and soft tissue.

Example 24

Administration and Imaging of Boiled ^{99m}Tc-HMDP

An Osteoscan® HDP kit is prepared as in Example 21. The ^{99m}Tc-HMDP is administered to a patient at a dosage of 20 mCi. Scintigraphic skeletal images of the patient are obtained approximately one hour post injection. The skeletal images are of good quality and suggest that the ^{99m}Tc-HMDP cleared rapidly from blood and soft tissue.

From the foregoing, it will be appreciated that the present invention provides technetium-99m mono-, di- and polyphosphonate compositions which clear rapidly from the blood and soft tissue to allow scanning of the patient in less than 4 hours post-injection and to lower the radiation dose to non-target tissues.

The invention may be embodied in other specific forms without departing from its spirit or essential characteristics. The described embodiments are to be considered in all respects only as illustrative and not restrictive. The scope of the invention is, therefore, indicated by the appended claims rather than by the foregoing description. All changes which come within the meaning and range of equivalency of the claims are to be embraced within their scope.

What is claimed is:

CLAIMS:

1. A method of diagnostic skeletal imaging using a technetium-99m skeletal imaging agent which rapidly clears from blood and soft tissue comprising the steps of:

(a) combining a mono-, di-, or polyphosphonate ligand with a radioactive pertechnetate ^{99m}Tc solution in the presence of a reductant and optionally a stabilizer at a pH in the range from about 1 to about 10;

(b) heating the mixture to a temperature in the range from about 50°C to about 150°C;

(c) placing the mixture in a pharmaceutically acceptable carrier to form a skeletal imaging agent having a pH in the range from about 2 to about 10;

(d) administering the skeletal imaging agent to a patient; and

(e) performing scintigraphic skeletal imaging of the patient within about four hours after administration.

2. A method of diagnostic skeletal imaging as defined in claim 1, wherein the mixture is heated in an autoclave.

3. A method of diagnostic skeletal imaging as defined in claim 1, wherein the mixture is heated in a microwave oven.

4. A method of diagnostic skeletal imaging as defined in claim 1, wherein the mixture is heated in a boiling water bath.

5. A method of diagnostic skeletal imaging as defined in claim 1, wherein the mixture is heated by sonication in a ultrasonic bath.

6. A method of diagnostic skeletal imaging as defined in claim 1, wherein the mixture is heated for at least about 1 minute.

7. A method of diagnostic skeletal imaging as defined in claim 1, wherein the mixture is heated for at least about 5 minutes.

8. A method of diagnostic skeletal imaging as defined in claim 1, wherein the mixture is heated to a temperature of at least 75°C for a period of from about 5 to about 60 minutes.

9. A method of diagnostic skeletal imaging as defined in claim 3, wherein the microwave oven has a power in the range from about 300-750 watts.

10. A method of diagnostic skeletal imaging as defined in claim 3, wherein the microwave oven has a power in the range from about 300-500 watts.

11. A method of diagnostic skeletal imaging as defined in claim 3, wherein the mixture is heated from about 10 seconds to about 5 minutes.

12. A method of diagnostic skeletal imaging as defined in claim 3, wherein the mixture is heated from about 30 seconds to about 2 minutes.

13. A method of diagnostic skeletal imaging as defined in claim 5, wherein the mixture is sonicated for a period from about 1 to about 30 minutes.

14. A method of diagnostic skeletal imaging as defined in claim 5, wherein the mixture is sonicated in combination with heating in a hot water bath for a period from about 1 to about 10 minutes.

15. A method of diagnostic skeletal imaging as defined in claim 1, wherein the scintigraphic skeletal imaging of the patient is performed within about three hours after administration.

16. A method of diagnostic skeletal imaging as defined in claim 1, wherein the scintigraphic skeletal imaging of the patient is performed within about two hours after administration.

17. A method of diagnostic skeletal imaging as defined in claim 1, wherein the scintigraphic skeletal imaging of the patient is performed within about one hour after administration.

18. A method of diagnostic skeletal imaging as defined in claim 1, wherein the phosphonate ligand is selected from methanediphosphonic acid (MDP), Methane-hydroxydiphosphonic acid (HMDP), ethane-1-hydroxy-1,1-diphosphonic acid (HEDP), N,N-dimethylaminomethane-diphosphonic acid (DMAD), propane-2,3-dicarboxy-1,1-diphosphonic acid (DAD), or pharmaceutically acceptable salts thereof.

19. A method of diagnostic skeletal imaging as defined in claim 1, wherein the reductant is a stannous salt.

20. A method of diagnostic skeletal imaging as defined in claim 1, wherein the mixture includes a stabilizer selected from ascorbic acid, gentisic acid, erythorbic acid, reductic acid, nicotinic acid, and pharmaceutically acceptable salts, esters, and amides thereof.

21. A method of diagnostic skeletal imaging as defined in claim 1, wherein the mixture has a pH in the range from about 1 to about 5 during the heating step.

22. A method of diagnostic skeletal imaging as defined in claim 1, wherein the diagnostic skeletal imaging agent has a pH in the range from about 4 to about 8.

23. A diagnostic skeletal imaging agent which rapidly clears from blood and soft tissue prepared by the process comprising the steps:

(a) combining a mono-, di-, or polyphosphonate ligand with a radioactive pertechnetate ^{99m}Tc solution in the presence of a reductant and optionally a stabilizer at a pH in the range from about 1 to about 10;

(b) heating the mixture to a temperature in the range from about 50°C to about 150°C; and

(c) placing the mixture in a pharmaceutically acceptable carrier to form a skeletal imaging agent having a pH in the range from about 2 to about 10, wherein the diagnostic imaging composition is stable over time as evidenced by HPLC analysis.

24. A diagnostic skeletal imaging agent as defined in claim 23, wherein the mixture is heated in an autoclave.

25. A diagnostic skeletal imaging agent as defined in claim 23, wherein the mixture is heated in a microwave oven.

26. A diagnostic skeletal imaging agent as defined in claim 23, wherein the mixture is heated in a boiling water bath.

27. A diagnostic skeletal imaging agent as defined in claim 23, wherein the mixture is heated by sonication in a ultrasonic bath.

28. A diagnostic skeletal imaging agent as defined in claim 23, wherein the phosphonate ligand is selected from methanediphosphonic acid (MDP), Methane-hydroxydiphosphonic acid (HMDP), ethane-1-hydroxy-1,1-diphosphonic acid (HEDP), N,N-dimethylaminomethane-diphosphonic acid (DMAD), propane-2,3-dicarboxy-1,1-diphosphonic acid (DAD), or pharmaceutically acceptable salts thereof.

29. A diagnostic skeletal imaging agent as defined in claim 23, wherein the reductant is a stannous salt.

30. A diagnostic skeletal imaging agent as defined in claim 23, wherein the mixture includes a stabilizer selected from ascorbic acid, gentisic acid, erythorbic acid, reductic acid, nicotinic acid, and pharmaceutically acceptable salts, esters, and amides thereof.

31. A diagnostic skeletal imaging agent as defined in claim 23, wherein the mixture has a pH in the range from about 1 to about 5 during the heating step.

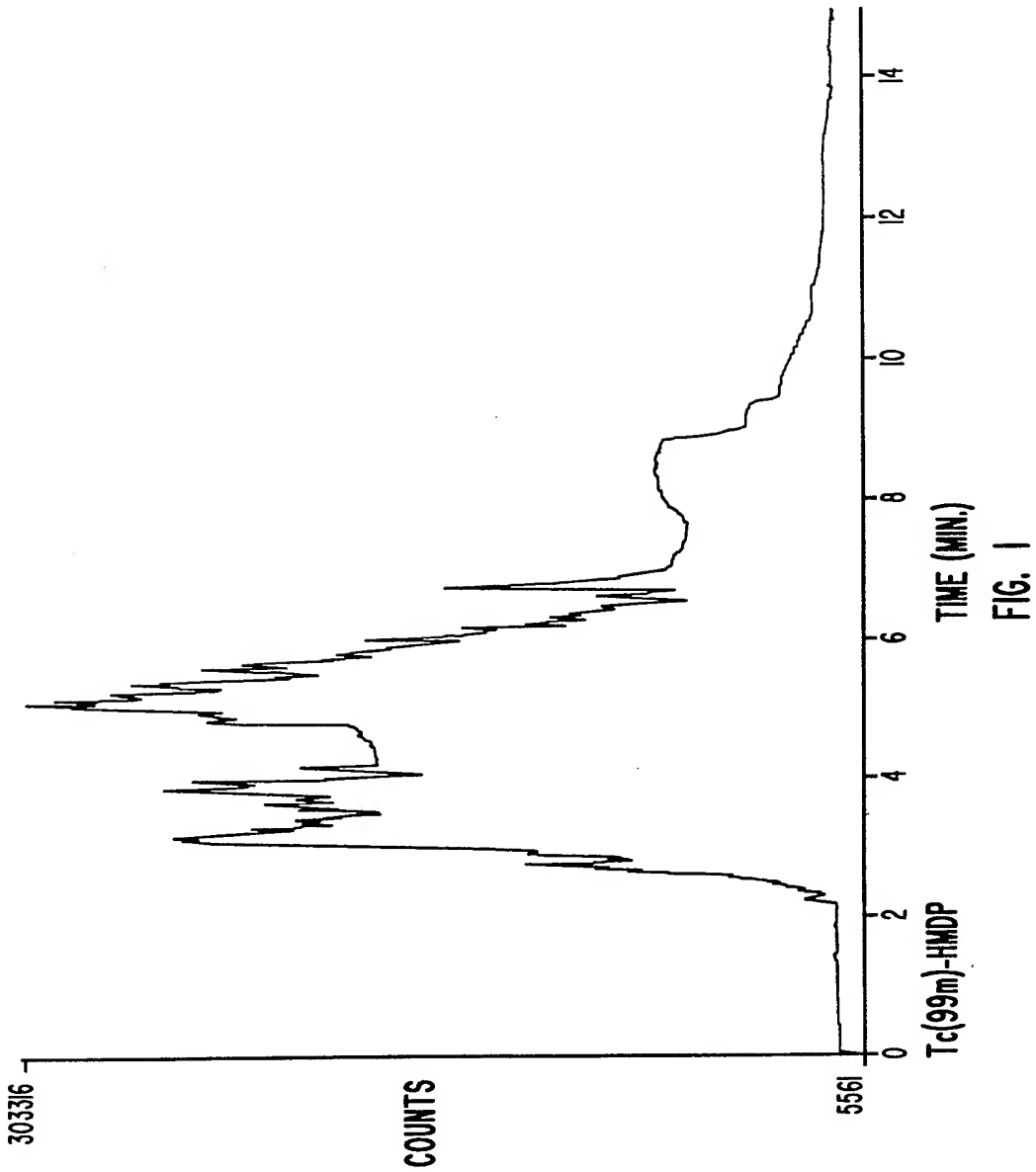


FIG. 1

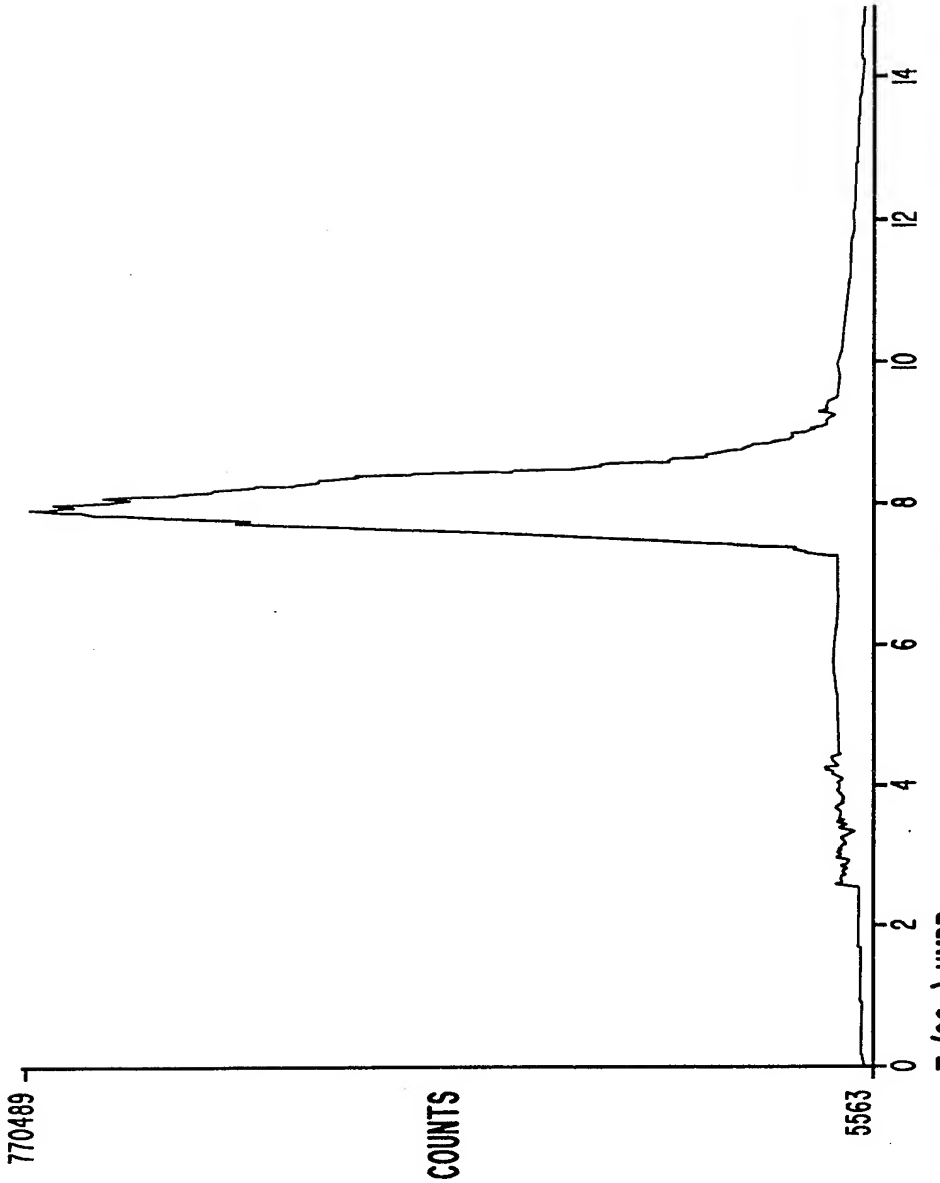


FIG. 2

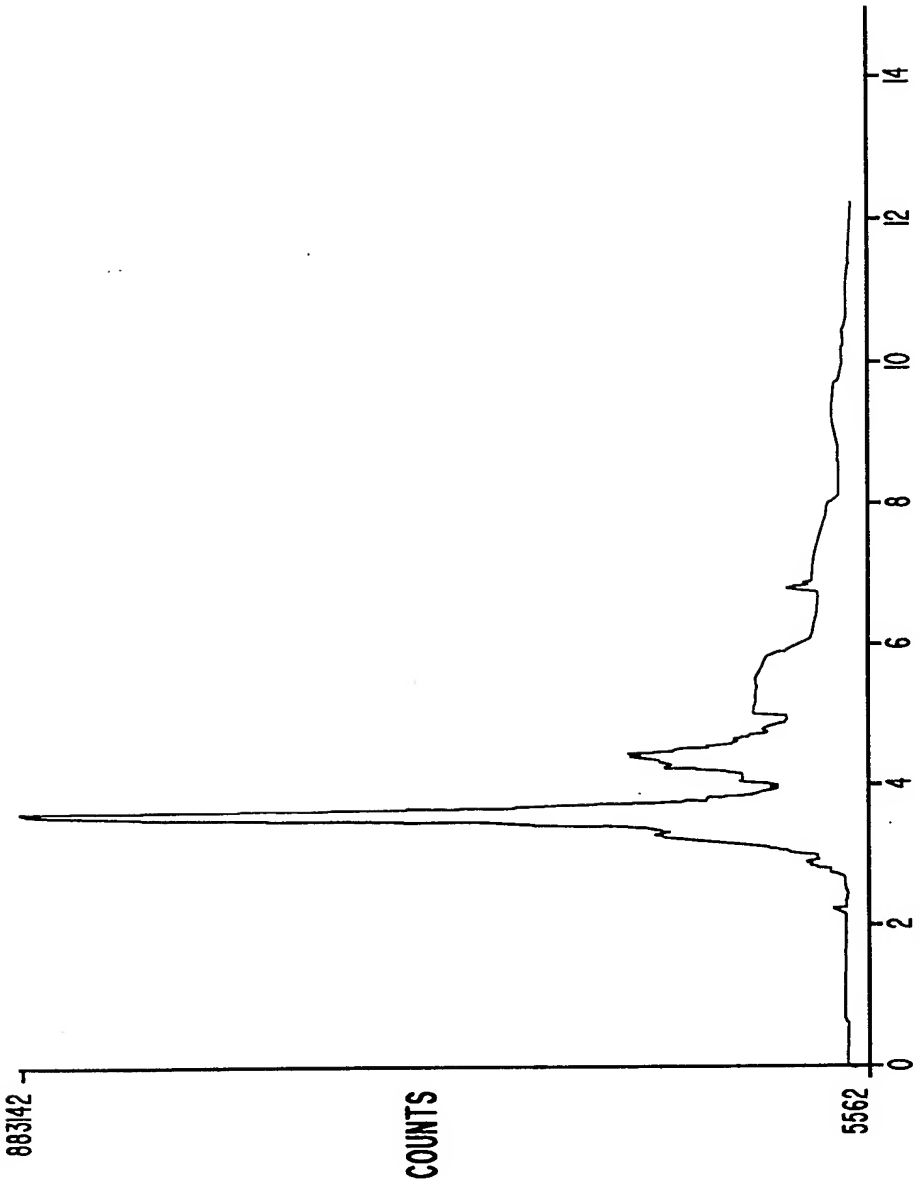


FIG. 3

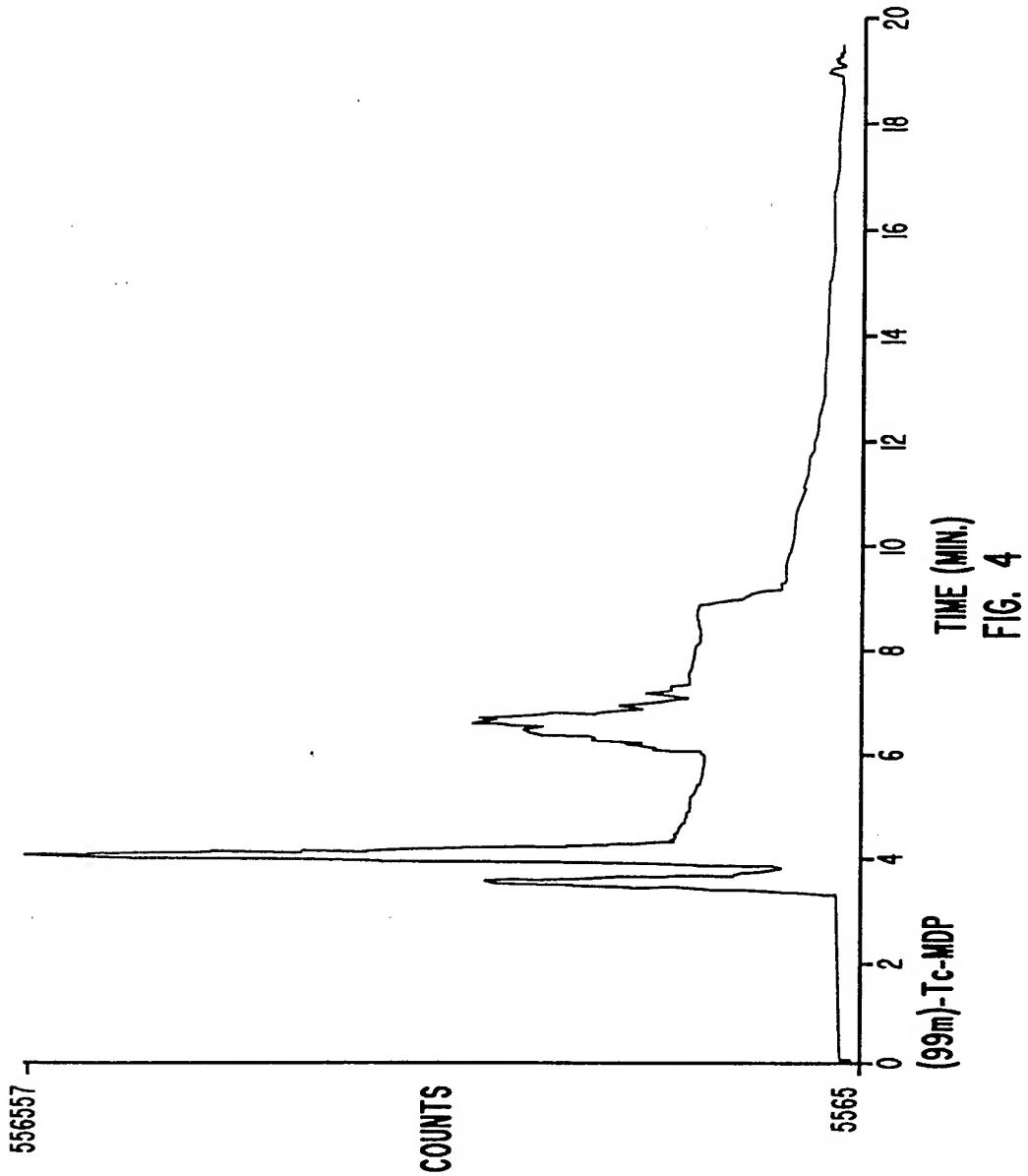


FIG. 4

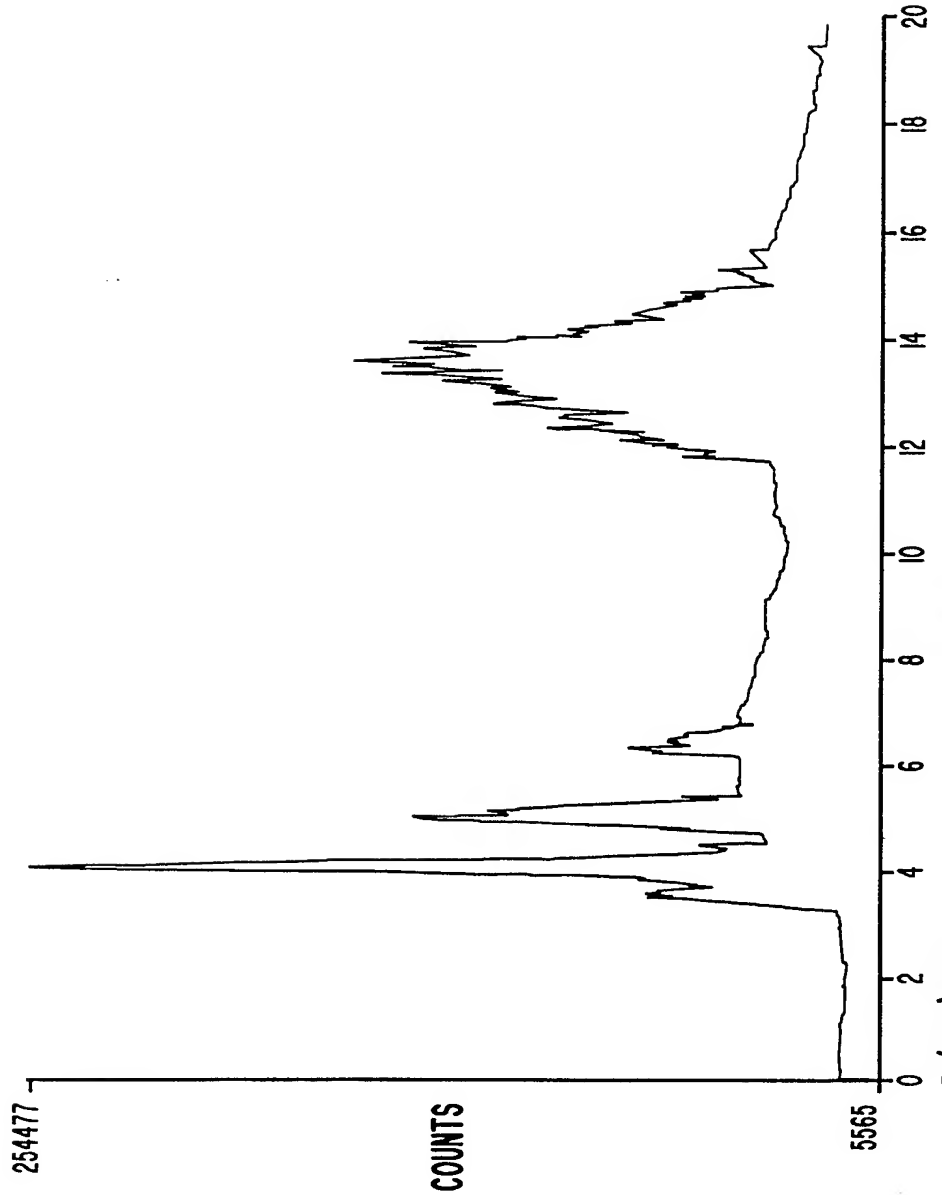


FIG. 5

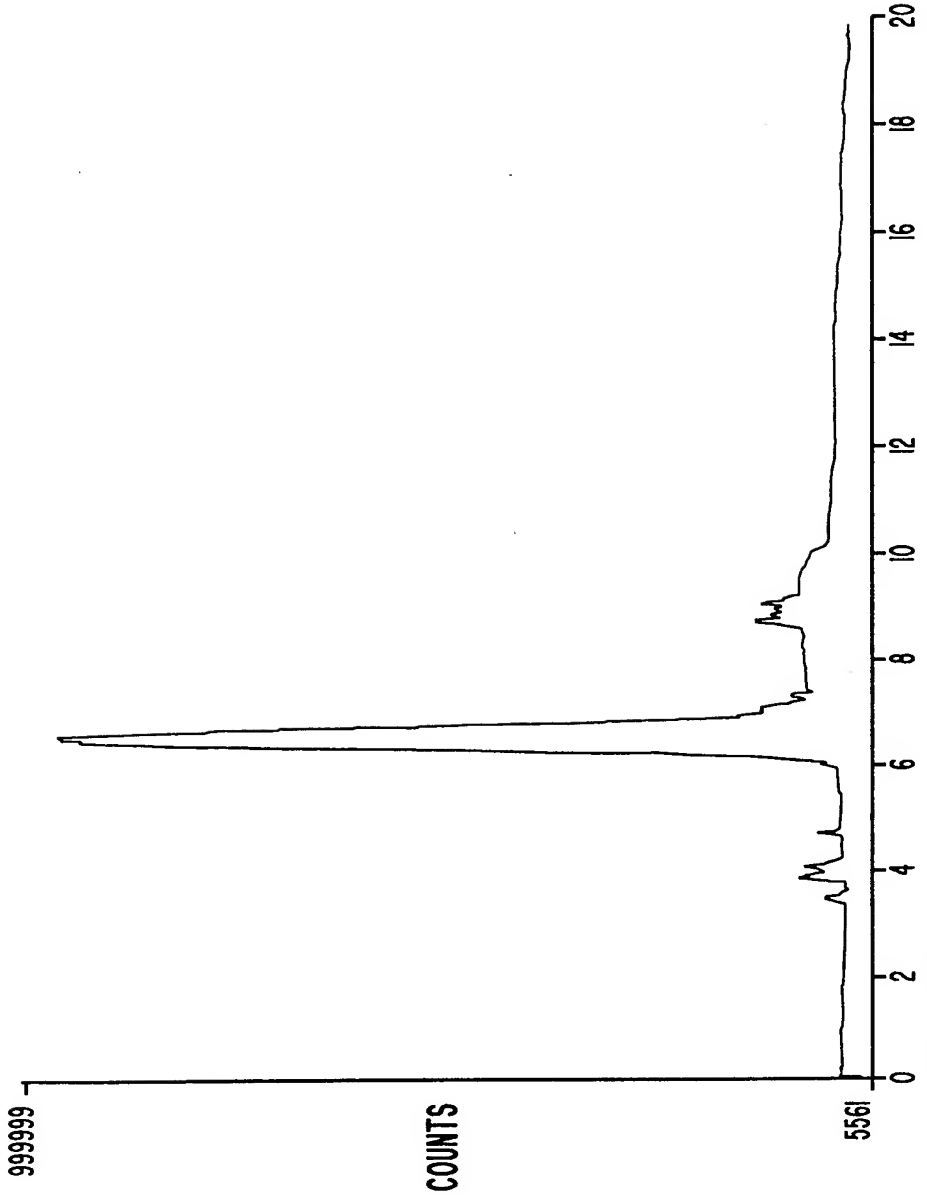


FIG. 6

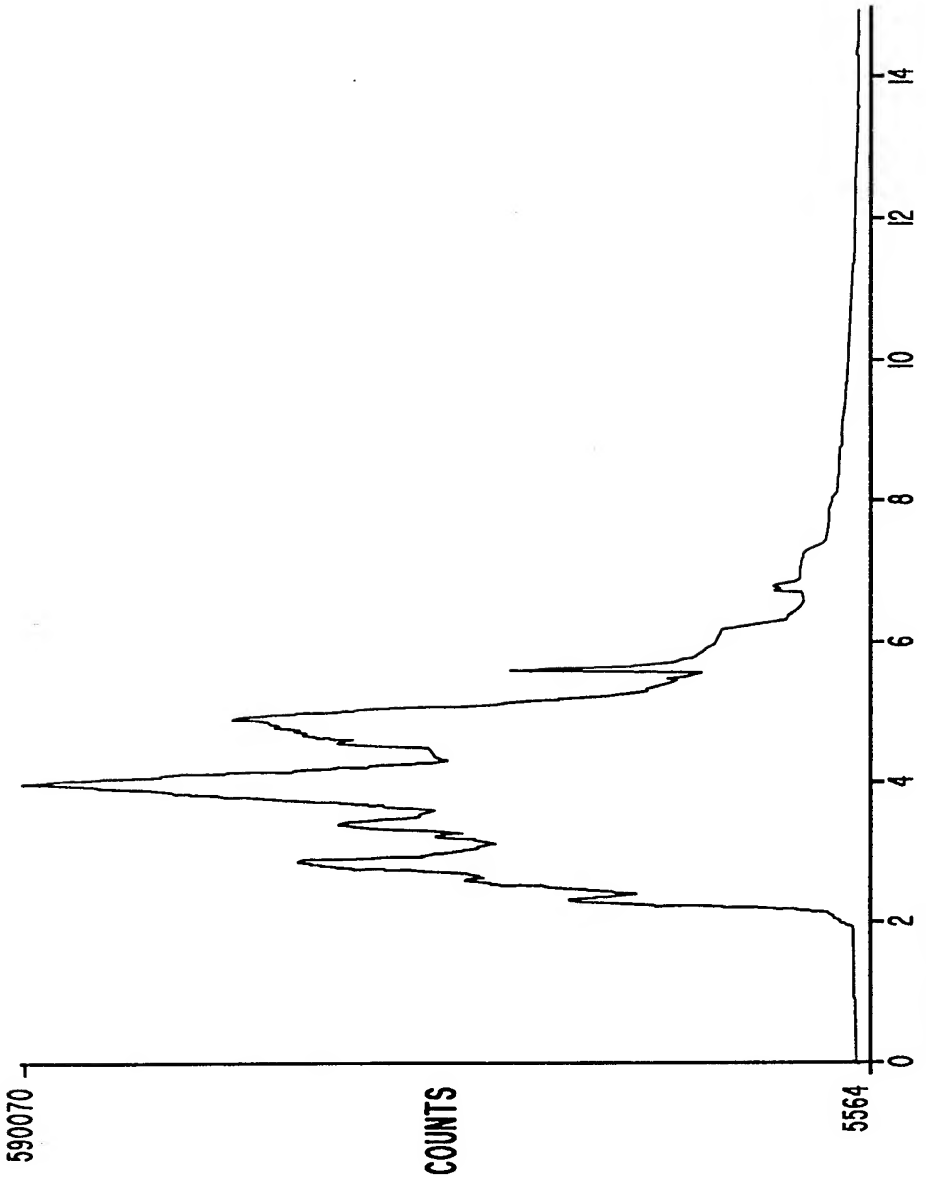


FIG. 7

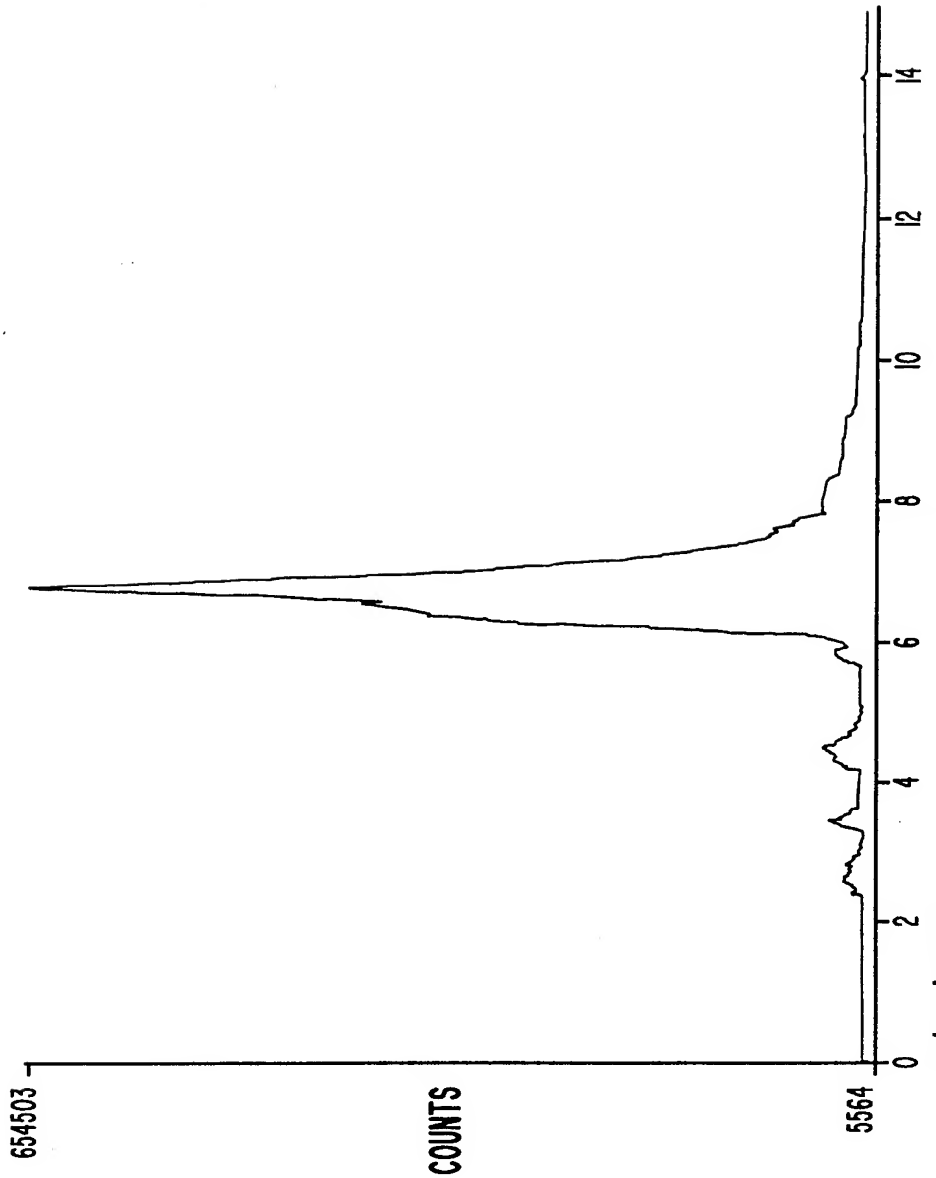


FIG. 8

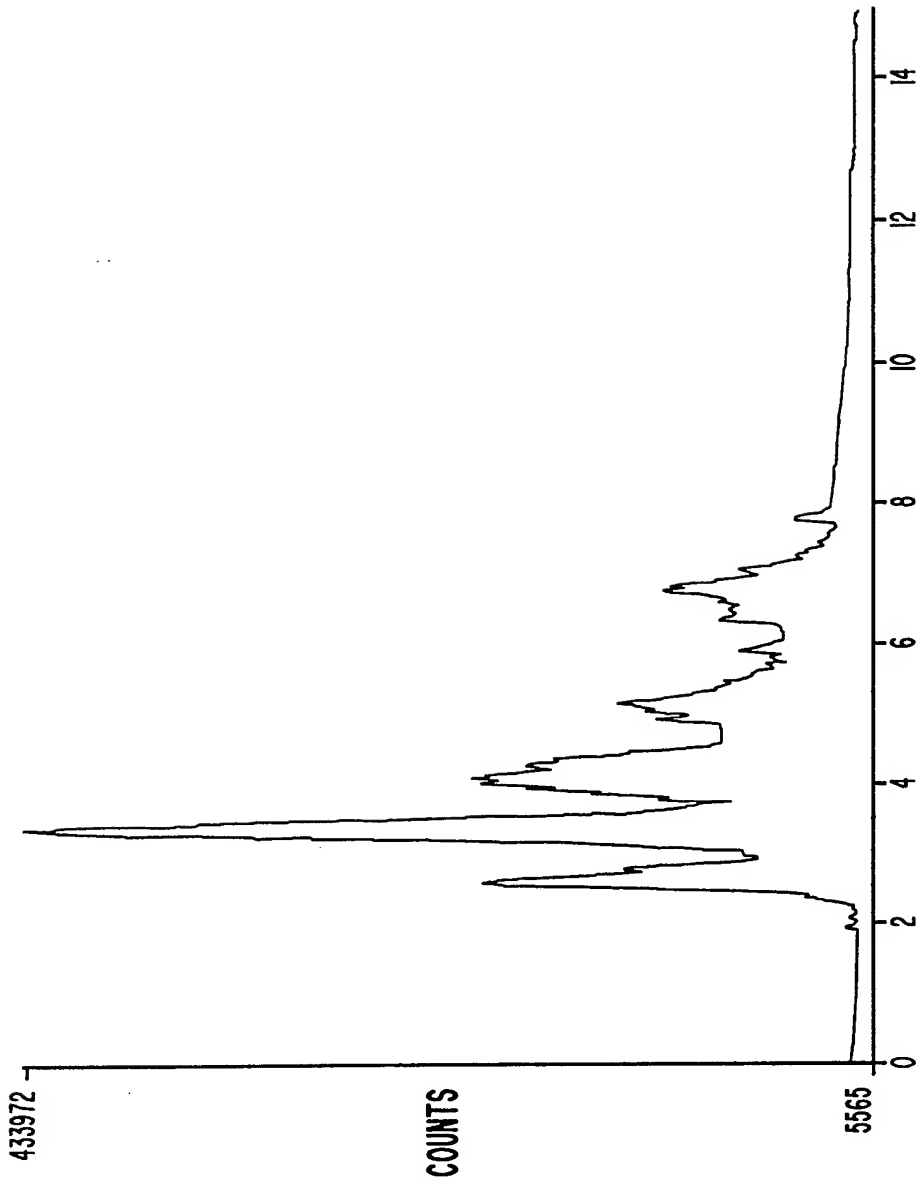


FIG. 9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/06276

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :CO7F 13/00; A61K 49/02
US CL :534/10, 14; 424/1.77
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 534/10, 14; 424/1.77

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US, A, 5,089,249 (FRITZBERG ET AL.) 18 February 1992 see columns 2-5.	23-31 --- 1-22
X	THIRD WORLD CONGRESS OF NUCLEAR MEDICINE AND BIOLOGY, 1982, SRIVASTAVA ET AL., "HPLC Characterization of Clinically Used 99m Tc Bone Agents," pp. 1631-1634. see entire document.	23-31
X	INT. J. APPL. RADIAT. ISOT. VOL 33, 1982, PINKERTON ET AL., "In Vivo Distributions of some Component Fractions...," pp. 907-915. See entire document.	23-31
Y	WO, A, 92/00758 (MALLINCKRODT) 23 JANUARY 1992. See entire document.	1-31

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 29 SEPTEMBER 1994	Date of mailing of the international search report 03NOV 1994
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